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Unravelling the physiological basis of superficial scald in pears based on cultivar differences

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ABSTRACT

Superficial scald is an important physiological disorder affecting both apple and pear fruit during postharvest storage. To date, superficial scald has been associated to many different preharvest and postharvest factors which are ultimately affected by the genetic characteristics of each cultivar. Accordingly, this work investigated differences in scald susceptibility during cold storage in two different pear cultivars 'Beurré d'Anjou' and 'Packham Triumph' and its relation to the changes in ethylene production, accumulation of α-farnesene and in its oxidation products (CTols), and finally changes in the fruit antioxidant potential and ascorbate levels.

Collectively the results from this study indicate that superficial scald in pear develops differently than in apples. The highest sensitivity observed in 'Beurré d'Anjou' pears was not related to ethylene and/or to the capacity of the fruit to accumulate α-farnesene, but rather to its capacity to prevent the accumulation CTols. Although presenting similar values in global antioxidant potential, the higher resistance of 'Packham Triumph' pears to superficial scald was positively associated to higher ascorbate levels. The potential involvement of ascorbate in preventing superficial scald development is further discussed.

1. Introduction

Despite many years of research, the biochemical or physiological mechanisms underlying superficial scald disorder in pome fruit are still debatable. It is generally accepted that scald is the result of an oxidative process (Lurie et al., 2005) in which α-farnesene (AF) and its oxidation products, the conjugated trienols (CTols), play a predominant role (Huelin and Murray, 1966; Anet, 1972; Whitaker, 2007; Whitaker et al., 2009). For decades, this model has been supported by different observations such as: 1. the beneficial effects of ventilation and oiled wraps on AF content and scald (Huelin and Coggiola, 1968); 2. the effects that the inhibitors of isoprenoid biosynthesis or ethylene (1-MCP) have on α-farnesene production and scald development (Ju and Curry, 2000a, 2000b); and 3. the induction *in vivo* of scald disorder through the application of exogenous CTols (Rowan et al., 2001). This model was recently confirmed by Pechous et al. (2005) and Gapper et al. (2006), which show in both apples and pears that the inhibition of α-farnesene synthesis by 1-MCP was closely correlated with the suppression of the alpha farnesene synthase (AFS1) gene activity.

Despite of this body of evidence, there are also different reports that contradict this model. The most notable was cited in the work of Rao et al. (1998) carried out in 'White Angel' × 'Rome Beauty' hybrid apple lines in which scald disorder was not related to AF and CTols but rather to peroxidation, peroxidase and catalase activities. Whitaker et al. (2000) also supported this finding on the same experimental model and found that oxidation products of α-farnesene are not required for induction of scald but rather in worsening the symptoms in fruit already compromised by oxidative stress. Finally in our recent work carried out in 'Beurré d'Anjou' pears we also hypothesized that AF and CTols are less responsible for scald development than antioxidants in pears (Calvo et al., 2015).

Given that superficial scald is considered the result of an oxidative process, it is accepted that this disorder depends on the balance between the content of oxidative species and antioxidants within the fruit skin (Ju et al., 1996; Rao et al., 1998; Diamantidis et al., 2002; Zubini et al., 2007; Whitaker et al., 2009; Silva et al., 2010).

Accordingly, several authors have studied the influence that some specific antioxidants (i.e. p-coumaryl fatty-acid esters, also

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referred as CT_{258} substances) have on scald development in apples (Du and Bramlage, 1993; Whitaker, 1998) but with diverse results depending on the cultivar. Rudell et al. (2009) established some correlations between α-tocopherol degradation and an increase of superficial scald incidence during storage. Although these results may indicate an action of α-tocopherol in scald prevention, this hypothesis was contradicted when this substance was applied exogenously to fruit (Anet and Coggiola, 1974; Ju et al., 2000). The activity of other water-soluble antioxidants has also been investigated. In this context, water-soluble antioxidant concentrations declined during storage and no individual antioxidant was associated consistently with scald or CTols accumulation (Barden and Bramlage, 1994).

All these findings warrant further consideration of the importance of genetic regulation of resistance to superficial scald. In this way, the differences that may exist between different cultivars may be considered a valuable source of information to better understand the physiological basis of superficial scald in pear. Accordingly, the present study was conducted on 'Beurré d'Anjou' and 'Packham Triumph' pears to evaluate metabolic differences that could explain the disparity in superficial scald susceptibility exhibited among these two cultivars. Emphasis was given on the rates of ethylene production, accumulation of α-farnesene (AF) and its oxidation products and finally to the levels of antioxidants and ascorbate during storage in cold air.

2. Material and methods

2.1. Plant material and storage conditions

'Beurré D'Anjou' and 'Packham Triumph' pears (*Pyrus communis* L.) were harvested from a commercial orchard located in Alto Valle, Rio Negro, Argentina from 10 years old trees, planted at 4 × 2 m, on seedling rootstock trained in a modified Trellis system.

Fruit were picked of uniform size and free from defects at optimum harvest date the 10th February and 18th February for 'Beurré D'Anjou' and 'Packham Triumph' pears respectively.

Immediately after harvest, fruit were transferred to the laboratory and packed in cardboard boxes with two trays (20 fruit each), that were stored in regular air at −0.5 °C and 95% RH for 240 days.

2.2. Fruit quality determinations at harvest

Quality parameters were determined on 5 replicates of 10 fruit each. Flesh firmness (N) was measured using a fruit texture analyzer (FTA-GS14, Güss, South Africa), with an 8 mm diameter plunger. Two measurements were carried out per fruit on opposite sides, after removal of 2 mm of peel. Soluble solid content (SSC) and titratable acidity (TA) were determined using freshly prepared juice from each individual fruit. SSC (%) was measured using a temperature-compensated digital refractometer (Pal1, Atago, Japan) and TA ($g\,L^{-1}$) expressed as malic acid content was measured by titrating 10 mL juice with 0.1 N NaOH to an endpoint of pH 8.2, using a calibrated pH meter (Bicasa, B.E 105, Italy). The percentage of starch degradation (%) was determined by comparison to specific tables (INTA editions 2008, Argentina) for each variety after inserting a fruit slice of 1–1.5 mm from the equatorial zone in a lugol solution.

2.3. Ethylene measurements

Ethylene production was measured in 5 replicates of one fruit each after 0, 15, 30, 45, 60, 90, 120, 150,180, 210 and 240 days at −0.5 °C and during 30 days shelf life at 20 °C. At each time of analysis, fruit were sealed in 1.5 L airtight jar for 30 min. Gas samples of 1 mL were extracted with a syringe from the air headspace. The sample was analysed with a gas chromatograph (GC14-A, Shimadzu, Japan) equipped with an FID detector, an activated alumina column and injector operating at 240 °C, 40 °C and 110 °C respectively. Helium was used as carrier gas. Ethylene production curves were drawn to determine the value of maximal ethylene production during the self-life (nL/g h).

2.4. Determination of superficial scald incidence

Scald incidence was estimated visually after 60, 90, 120, 150, 180, 210 days at −0.5 °C and 7 additional days of commercial life at 20 °C. At each time the number of damaged fruit (% fruit with scald symptoms) and the severity of the symptoms was determined on 5 replicates of 10 fruit as described elsewhere (Calvo et al., 2015).

2.5. Determination of α-farnesene (AF) and conjugated trienols (CTols)

AF and CTols were analysed in 10 replicates of one fruit each, after 0, 15, 30, 45, 60, 90, 120, 150,180, 210 and 240 days at −0.5 °C following the method described by Anet (1972), with some modifications (Calvo et al., 2015). At each removal time, a strip of peel of 2 mm thick was removed from the equatorial zone of each fruit and 5 discs (10 mm diameter) discs prepared using a cork borer. The discs were then immersed in 10 mL of HPLC grade hexane for 10 min with constant stirring. 1 mL of this solution was diluted in 4 mL of hexane and used for analysis.

Measurements were performed calibrating first the equipment with HPLC grade hexane. Absorbance at 232 nm (α-farnesene) and 281–290 nm (conjugated trienols) were recorded using a UV-spectrophotometer (1001 Plus, Milton Roy, USA). Concentrations of α-farnesene and conjugated trienols were calculated using the molar extinction coefficients E_{232} nm = 27,700 for α-farnesene and $E_{281-290}$ nm = 25,000 for conjugated trienols (Anet, 1972) and expressed as nmol cm^{-2} of fruit.

2.6. Quantification of total antioxidant capacity

Total antioxidant capacity was determined using the DPPH test. Measurements were performed on 5 replicates of 10 fruit each, after 0, 15, 45, 60, 90, 120, 150, 180, 210 and 240 days at −0.5 °C. The entire peel of the fruit was removed, frozen with liquid nitrogen, lyophilized and ground to a fine powder that was homogenized with 10 mL of 80:20 methanol:water (v/v). Samples were left for 2 h at room temperature in a constant shaking bath, and then centrifuged at 20 °C (24,000*g*) for 15 min. The supernatant obtained was then filtered and diluted with milli-Q water (1:4; v/v). A 20 μL aliquot of the diluted extract was then mixed and stirred with 980 μL 1-diphenyl-2- picrylhydrazyl (DPPH; Sigma Aldrich, Steinheim, Germany) in the dark for 30 min at 4 °C.

Initial absorbance (Ai) was measured at 517 nm on the blank and final absorbance (Af) was measured after the incubation period using a UV-spectrophotometer (1001 Plus, Milton Roy, USA) following calibration with double distilled water. Inhibition (%) was calculated as follow: (Ai) − (Af)/(Ai) * 100. Under these conditions, an increase in value corresponded to increase in the total antioxidant activity.

2.7. Determination of ascorbate levels

Total ascorbic acid was extracted following the protocol of Misra and Seshadri (1967) on 6 replicates of 5 fruit each. Samples were obtained mixing the skin of each replicate with 300 mL 1% (p/v) H_3PO_4 and homogenizing the resulting solution with a homogenizer. 0.15 mL of sample were added to 0.5 mL of 30 mg L^{-1} 2,6-diclorofenol-indofenol and incubated during 30 s. All

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